SERUM PROTEINS AND IMMUNOGLOBULIN LEVELS IN CHILDHOOD TUBERCULOSIS

THESIS
FOR
DOCTOR OF MEDICINE
(PAEDIATRICS)

BUNDELKHAND UNIVERSITY
JHANSI [U. P.]

1993 **Prem Prakash Gupta**

C E R T I F I C A T E

This is to certify that the work entitled "SERUM PROTEINS AND IMMUNOGLOBULIN LEVELS IN CHILDHOOD TUBERCULOSIS" has been conducted by PREM PRAKASH GUPTA in the Department of Paediatrics, M.L.B. Medical College, Jhansi.

He has put in the necessary stay in the department according to university regulations.

Dated : 31.3.1993.

31/3/93

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C E R T I F I C A T E

Certified that the work entitled "SERUM PROTEINS AND IMMUNOGLOBULIN LEVELS IN CHILDHOOD TUBERCULOSIS" has been conducted by PREM PRAKASH GUPTA, under my guidance and supervision in the department of Paediatrics, M.L.B. Medical College, Jhansi.

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I N D E X

ACKNOWLEDGEMENT

ACKNOWLEDGEMENTS

A task such as this, could not be carried through and completed without the support of my teachers, colleagues and others, too numerous to mention whose help, I gratefully acknowledge.

I express my deepest regards to my guide, Dr. Ramesh Kumar, M.D., D.C.H., Professor and Head, department of Paediatrics, M.L.B. Medical College,Jhansi, whose uncompromising standards, generousness, and ever patronizing attitude had always been and will be ever a source of encouragement to me.

I don't find the words to convey my sincere most thanks to Dr. (Mrs.) Sheela Longia, M.D., Reader, Department of Paediatrics, M.L.B. Medical College, Jhansi. Her co-operative and sympathetic attitude had always helped to boost up my morale during periods of frustation.

I wish to express my sincere gratefulness to my teacher Dr. Anil Kumar Kaushik, Assistant Professor, Department of Paediatrics, M.L.B. Medical College, Jhansi, whose unfathomed knowledge, elegant endeavour and ingenious efforts are associated with this humble venture.

I would also like to thank Dr. R.S. Sethi, M.D., D.C.H., Assistant Professor, Department of Paediatrics, M.L.B. Medical College, Jhansi for encouraging and helpful attitude. His suggestions had been very valuable during the states of dilemma.

I owe my whole of the carrier to my mother and other family members without their help I would have never achieved the present status. I am proud of them, and my head as a mark of respect.

I gratefully acknowledge the undefinable help and support extended to me, by my colleagues and friends. I shall always remain indebted to them.

I am also thankful to Mr. R.S. Vishwakarma for his meticulous care and painstaking labour in preparing the type script of this project.

Dated: 31.3. 1993.

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INTRODUCTION

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Tuberculosis is distributed throughout the world. But its prevalence varies greatly. A global review (1961-1971) of tuberculosis by WHO showed that tuberculosis is a major health problem in developing countries. With an annual rate of fresh infection at 4% in children of India (Narayan 1980), childhood tuberculosis in India constitutes a problem of tremendous magnitude, compounded and perpetuated by large pool of infections adults. Almost 1.5 million new cases of adult chronic pulmonary tuberculosis are detected annually in India. With such a high pool of infectious cases, nearly 1.9 million children under the age of 5 years get primary infection annually (Udani 1983).

The estimated prevalence of infection in 0-4 years group is 2.1% (Goth 1974). From the study carried out in Chingle-put region, 4 years after BCG trial started, the incidence of infection was 2.8% in 0-4 years age group, 4.4% in 5-9 years and 5.8% in the age group of 10-14 years (Tripathi 1982). Applying this incidence of infection, there would be 2.6 million infected children in the age group of 0 to 4 years, 3.9 million, in 5 to 9 years age and 4.9 million in 10-14 years age group.

The prevalence of infection in a total child population, both rural and urban areas, between the age of 1 to 14 years is 16% (Tripathi 1982).

On the basis of criteria laid down by WHO no single country in the world has succeeded in reaching the point of control i.e. less than 1% tuberculin positivity among children in the age group of 0-14 years.

Tuberculosis in infancy and childhood poses serious problem particularly because of its high morbidity and mortility, vague symptomatology and chances of getting miliary and disseminated tuberculosis. Therefore, it deserves special emphasis and its early diagnosis and prompt treatment is very important. Despite all microbiological advances the diagnosis of an active case of tuberculosis is still far from satisfactory.

The immunology of tuberculosis has been intensively studied by numerous workers since Robert Koch's pioneering work in 1891. Until recently, cell mediated and humoral (antibody dependent) immune responses were often regarded as separate and independent phenomenon. But now, it is realized that all immunological reactions are closely

inter-related. The outcome of an infection, such as tuberculosis, is not a single protective response but a mean of several responses.

The principal immune response in tuberculosis is cell mediated type. However, it has been established by various workers that there is a definite antibody response in human beings. The level of three immunoglobulins viz IgG, IgA and IgM is raised in tuberculosis as compared to control cases (Singh et al 1984).

In the immune spectrum of tuberculosis, in adult, an exaggerated antibody response with poor cell mediated response is indicative of unreactive, markedly diffuse leisons in the chest with poor response to treatment (Lenzini et al 1977). Seth et al (1985) studied the clinical spectrum of tuberculosis in children and found that symptomatic mantoux positive (SMP) and primary pulmonary complex (PPC) cases are comparatively milder forms of disease. The level of IgG was significantly increased whereas IgM level was significantly decrease in children with PPC and SMP in comparison to controls.

The immune response in tuberculosis by the bacterial immunomodulatory factors as well as the pathogenetic mechanisms results in development of both -

(i) Immediate type of hypersensitivity, manifested by the production of circulating antibodies (B cell) mediated humoral immunity; and (ii) delayed type of hypersensitivity, manifested by the familiar tuberculin reaction (T cell) mediated cellular immunity. It has been prevalent belief that antibodies do not play a protective role in determining resistance to tuberculous disease. Nevertheless, the antibody response of tuberculous patients has been extensively studied since the turn of century, mainly with the objective of devising an effective, simple and reliable serodiagnostic test.

It is in the light of the observation that the present venture is directed to observe and confirm the various immunoglobulin levels (IgA, IgM, IgG) in childhood tuberculosis and its significance, if any, in the serodiagnosis in various forms of the disease.

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REVIEW OF LITERATURE

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HISTORY OF TUBERCULOSIS :

Tuberculosis is known to be one of the oldest human diseases, begun in antiquity. The study of tuberculosis has continued down the centuries and in unfolding has created its own monuments, each marked by the name of a man whose flash of the genius or whose dedication has helped to illuminate the way towards the ultimate goal "the conquest of the disease."

The Indo-aryans were aware of the disease as is obvious from Rigveda (1500 B.C.) and Ayurveda (700 B.C.). Hippocrates described tuberculosis for the first time and named the disease 'Phthisis' derived from the Greak word 'Phthein' i.e. waste away (460 to 375 B.C.).

Reirre Desault 1733 published an account of Phthisis maintaining that tubercles were new structures and the cause of phthisis was contagious through the agency of sputum.

Virchow (1847) described caseation and Langheu 1868 described the characteristic gaint cells of tuberculosis. Robert Koch (1882) announced the discovery of tubercle basilus and in 1890 he desclosed

to world his development of the tuberculin and pastulated his phenomenon. Later, at the turn of century in 1907 Pirquet described cutaneous reaction to tuberculin on the basis of allergy. In 1921, Calmette and Guarin laid a mile stone in the history of tuberculosis by developing B.C.G. vaccine.

Signs of pott's disease have been well established in nesprehan, a well preserved egyptian "Mummy" of 21st dynasty (Cave 1939). Prior to these developments tuberculosis was supposed to be a weak form of inflammation accuring without any specific cause and such a misconception gave rise to apathy in treatment (WHO 1962).

William Smith made the detailed study of structural changes in tuberculous lung (quoted by Keer's 1978).

MAGNITUDE OF PROBLEMS :

Tuberculosis even today remains a major health problem in the world, infecting about one billion people and causing an estimated 1-2 million deaths annually. In most of the developing countries, including India, the present risk of infection due to tuberculosis is 20 to 25 times higher than in developed countries (Styblo 1974).

PREVELANCE :

The prevelance of active disease in the population is about 15 to 25 per thousand population, 1/4 of them being bacillary or open cases of tuberculosis. Thus, out of the total estimated 813 million population, almost 15 million cases are bacillary or infectious (Pamra 1980).

The prevelance of primary infection in child population is very high. Nearly 3.4 million children have tuberculosis while 94 million are at risk of infection. In India 40% of children, by the age of 6 years and 80% by the age of 16 years, are labelled as infected (Udani 1983). Udani et al (1987) reported 3% annual rate of infection of tuberculosis and the difference between infection and disease in infants and young children under 3 years of age was not significant.

MORTALITY :

Even though accurate figures are not available mortality rates have declined in recent years. It is however, as high as 90 per ten thousand population in a rural area near Banglore (WHO Bulletin 1974).

Recently in another study while analysing the causes of death in 346 children, Dhadimal et al 1976 found that 16.46% of total deaths were due to tuberculosis of various types, of which T.B.M. accounted for 56.1%. In 1977 Thomas & Udani in a study of autopsy proved cases of deaths extending over a period of ten years, (both adults and children under 15 years) found that out of 7676 adults death, 11.6% were due to tuberculosis (mainly pulmonary form) while out of the 4080 autopsies conducted in children, 10.8% were due to various types of tuberculosis. Milliary tuberculosis and tubercular basal meningitis accounted for 39.1% of the total deaths.

Goyal et al in 1978 reported that mortality in urban areas was around 40 per thousand population.

THE TUBERCLE BACILLUS :

Mycobacterium tuberculosis belongs to genus mycobacterium. These occur in the form of rods ranging in size from 0.3 to 0.6 by 1-4 micrometer, straight or slightly curved. These occur singly and in occasional strands. In 1940 Anderson demonstrated that mycobateria were very rich in lipids constituting upto 40% of dry weight. According to Abramsons unlike other bacteria mycobacteria

grow very slowly in vitro, requiring a generation time of atleast 10 to 15 hours on the most favourable media (Youman & Youman 1949).

In 1977 Barksdale and Kim showed that mycobacteria possessed a thick lipoidal wall, constructed of several layers.

Goren & Brennan (1979) gave details of various components of mycobacterium as wax D (water soluble - adjuvants), N-glycorlyl muramyl dipeptide, trehalose 6,6 dimycolate (Cord factor), sulfatides, phthienoic acid, mycocerezic acid and C-mycosides.

Chaparas in 1982 demonstrated that cellwall randered the organism impermeable and the cell wall components participated in the induction of certain activities, some of which were helpful to the host as far as the containment of disease was concerned. He also commented that intact whole cell of mycobacterium was, for the most part, non toxic. However, the component parts could become toxic if the cell will broken-down, observed the author. He further opined that general adjuvanticity, granulomagenicity and capacity to activate macrophages and to increase the host resistance were associated properties and were induced by most of the components.

IMMUNOPATHOGENESIS OF TUBERCULOSIS - HUMORAL MECHANISM OF RESISTANCE :

Introduction of foreign antigen by natural infection or inoculation elicits number of reactions in the immune system, one of which is the production of antibodies. Quantitative and qualitative difference in humoral response is based on the genetic make up of animal, like the antigen degrading capacity of macrophages (Stievelman 1918). The antibody production usually requires co-operation between "B" and "T" lymphocytes and macrophages, resulting in proliferation and transformation of "B" cells into antibody secreting plasma cells (Grange et al 1984).

IMMUNOGLOBULINS :

Immunoglobulins are protein molecules that carry antibody activity. Antibodies arise in response to foreign substances introduced in the body. The immunoglobulins comprises a heterogenous group of proteins which account for approximately 20% of total plasma proteins. In serum electrophoresis, the majority of immunoglobulins migrate to the zone of designated γ - globulins. But significant amounts are also found in β - globulins zone.

A direct correlation between age and immunoglobins, IgGand IgM was seen upto 6 and 7 years respectively. No correlation was present between age and immunoglobulin concentration beyond 6 years in case of IgG and 7 years in case of IgA, suggesting that the adult concentrations of IgG and IgA were normally reached and maintained, after the age of 6 and 7 years respectively. In contrast analysis of the IgM data suggested that the adult value was reached by the age of one year. No significant difference was found in immunoglobulin concentrations which could be attributed to sex,(Buckley et al 1968).

BASIC STRUCTURE AND TERMINOLOGY :

Herdelberger and Pederson (1937) were the first who seperated immunoglobulins by size (the 195 fraction and 75 fraction). The larger fraction was named "immunoglobulin macro" or 'IgM' and smaller fraction "immunoglobulin gamma" or'IgG', a reflection of its electrophoretic mobility. Porter (1959) was able to cleave the immunoglobulin into two fragments, separable by ion exchange chromatography. One fraction retained the capability to react with immunogen and was called the antigenic binding fraction or Fab.

The other crystallized upon standing and was called the crystallizable fraction or 'Fc'.

Flerschman et al (1963) delineated the relationship between chain structure and proteolytic fragment and proposed a general model for immuno - globulin.

CLASS AND SUBCLASSES :

Five classes of immunoglobulin have been described in human, viz, IgG, IgA, IgM, IgD and IgE. IgG is further divided into four subclasses IgG1, IgG2, IgG3 and IgG4. Similarly two subclasses of IgA viz IgA1, and IgA2 have been clearly defined. Studies also indicate existence of two subclasses of IgM viz IgM1 and IgM2.

IgG :

This is most abundent immunoglobulin, 50% of its distribution is in the intravascular compartment. It has a molecular weight of 1,50,000 doltons.

Hardey et al (1969) observed that mean serum concentration of IgG in cord blood of newborn is usually in the range of 740-1650 mg% which consists of mainly maternal antibodies and falls to a level of about 200-600 mg% by six month of age . As the

infant is exposed to antigenic environment, IgG level gradually starts increasing, reacting to adult level (i.e. 800-1200 mg%) by about 4 years of age. The synthesis of IgG begins at the 11th week of gestation (Cocchi et al 1969 and Mc Cracken et al 1971).

IgA :

This immunoglobulin is found predominantly in external secreations of respiratory tract, gastro-intestinal tract, genitourinary system, tears and colostrum. The molecular weight is 4,00,000 doltons, composed of two IgA molecules held together by a single 'J' chain. Serum level of IgA is 0-3.9 mg% at birth and gradually increases to 25-75 mg% by the two years of age. The adult level (i.e. 150-300 mg%) is reached in adolescence (Malik et al 1977).

IgM :

Approximately 10% of the immunoglobulins are IgM, which is a pentamer of 9,00,000 molecular weight. The serum level of IgM in newborn is about 1.6 to 31 mg% which rapidly increases to adult level of 50-150 mg% by the age of one year (Hardey et al 1969).

HUMORAL MECHANISM OF RESISTANCE :

Antibodies against microbacterial agents have been demonstrated in IgG, IgM and IgA. Danial and Baum (1968 & 1969) have shown that antibodies to mycobacterium polysaccharide were principally in IgM class, while the antibodies agglutinating tuberculo-protein coated tanned erythrocytes were present in all the three classes.

Grange et al (1980) have emphasised that the antibodies in IgG class estimated by Elisa, using ultrasonicate of B.C.G. as the antigen, are diagnostically the most relevant. Authors observed that IgM antibodies were less discriminative. They opined that the primary humoral response to mycobacterium consisted of short lived IgM antibodies followed by predominance of IgG antibodies.

It is possible to isolate specific antibodies of mycobacterium tuberculosis, although a small fraction of antimycobacterial antibodies react with the specific antigen in tuberculosis (Grange 1982).

IMMUNE SPECTRUM IN TUBERCULOSIS :

The immunological parameters in human tuberculosis are of fundamental importance. Considering the general view that clinical type is determined by

a particular type of immune reaction rather than by the toxic products derived from the organism.

Skinsness in 1958 first suggested an immune response in tuberculosis in adults. Arakawa et al (1959) have classified tuberculous infected children into three types according to the presence of tuberculo-polysaccharide antigen and their antibodies in the blood. Nassau et al (1976) demonstrated the presence of antibodies measured by ELISA in 80% of patients with tuberculosis in comparison to 8% of healthy controls, while using a soluble filterate antigen from M. tuberculosis H37 RV.

Lanzini in 1977 established a spectrum of progressive human tuberculosis on clinical and immunological grounds.

Total IgG and IgA levels do not correlate well with the level of specific antibody but they have a correlation with the severity of disease in adults as demonstrated by radiological examination (Kardjito et al 1980). Grange et al (1980) have emphasised that the quantitation of specific antibodies in IgG and IgA is of greater diagnostic usefulness than an assay of total level of specific antibodies.

Daniel et al (1981) have reported the presence of high levels of antibodies against mycobacterial proteins in anergic patients. Winter & Cox (1981) have summarised that the antibody response to various antigens such as BCG, M tuberculosis whole cells, and BCG cell wall and PPD were not limited only to patients of active disease. Significant amount of antibody reactive with one or more of five antigens were demonstrated in 50% patients with inactive disease, 27% of the skin test positive healthy persons and 10% tuberculin negative healthy subjects.

Humoral antibody production by "B" lymphocytes is substantially affected in tuberculosis (Bates 1982). Cherunushenko and Colleagues (1982) have reported similar results in various forms of the pulmonary tuberculosis in adults.

Viljamen et al (1982) have attributed low IgM antibody concentration to :

(i) Tuberculosis being a chronic disease, it is possible that antibodies disappear early;

(ii) the adjuvant effect of mycobacteria results in a significant increase in total serum IgG and IgA but not IgM level; and

(iii) contact with mycobacterial antigens naturally or by BCG vaccination results in secondary type of humoral immune response in active tuberculosis without high IgM concentration.

SERUM PROTEINS AND IMMUNOGLOBULIN LEVELS IN TUBERCULOSIS :

Baldwin et al in 1952 studied serum proteins in human tuberculosis by electrophoretic technique. They found increase in alpha - 1 and alpha - 2 and gamma-globulins in pulmonary tuberculosis as the disease progressed. These changes were accompanied by the corresponding decrease in albumin. No significant changes were detected in the serum with minimal pulmonary tuberculosis.

In 1956 Gilliland et al studied serum proteins in pulmonary tuberculosis to determine the nature of change in serum proteins with particular reference to the possibility of a direct relationship existing with the extent, duration and severity of lesion. They studied the serum protein fractions by electrophoresis. There was a progressive fall in albumin concentration and a progressive rise in alpha-2 globulin concentration as the extent of lesion increased in pulmonary tuberculosis patients.

Leggat (1957) studied 18 cases of adult pulmonary tuberculosis, 9 of these cases were far advanced, 7 moderately advanced and 2 minimally affected cases. A common finding in all the eighteen cases was a very low initial serum albumin level and a high alpha-2 level, with low albumin/ alpha-2 ratio. There was a rapid fall in albumin wherever clinical deterioration occurred. The values returned towards normal over a period of months, as healing of the disease occurred. In 1961 Bovoronkitti studied 58 tuberculosis patients in the age group of 16 to 80 years. He estimated total serum proteins and analysed the electrophoretic pattern of these proteins, prior to treatment. The results demonstrated that there were definite changes in serum proteins from the normal values in active tuberculosis to an increase in alpha-2 globulins in all forms of the disease. Authors opined that increase in alpha-2 globulins occurred as a result of hypersensitivity reaction and changes in other components were related mainly to liver impairment. Increased amounts of gammaglobulin also denoted the presence of circulating antibodies in response to tuberculous infection.

Patnode et al (1966) studied serum protein changes in sarcoidosis in 29 adult patients and 19 normal adults as control. Immunoglobulin level in the serum of patients and controls were measured by means of "Agar ring diffusion technique". A statistically significant increase in IgG but not in IgA and IgM was seen in sarcoidosis patients.

Faulkner et al (1967) studied the alteration in immunoglobulin levels and serum proteins in 38 adult patients, who were sputum positive for M. tuberculosis, using immuno diffusion plates. They found elevated levels of IgG and IgA whereas IgM level was not altered. IgG and IgM levels did not correlate well with eachother. Authors observed lower albumin and elevated alpha-2, Beta-2 and gamma globulin levels in the sera of patients as compared to control cases. All the serum protein fractions in the sera of control group were significantly different with respect to the race. Lower serum albumin and higher globulin levels were observed in Negro race. The IgG levels in Negro sera were generally higher than that observed in Caucasion sera.

In 1970 Malomo studied serum immunoglobulins of 201 patients suffering from pulmonary tuberculosis in an age group of 7 months to 75 years, by single radial immuno-diffusion technique. Although, all the three immunoglobulins showed a considerable rise, increase in IgA level of the most significant. This difference was more pronounced in sputum positive cases; the highest value was atleast four times the upper limit of normal value. Lungs being the site of lesion in patients and are known to contain a high population of IgA producing immune cells could be responsible for producing high IgA levels, as observed by the author. The increase in three immunoglobulins could be attributed to the multiplicity of antigens in the tubercle baccilus, as opined by the author.

Bardana et al (1972) studied 86 patients of pulmonary tuberculosis. The study was carried out to detect and quantify humoral antibodies to components derived from M.tuberculosis, using both quantitative and qualitative primary tests. The binding was observed by sera obtained from all normal as well as from tuberculous patients. There was however, a lower amount of binding in sera obtained from control cases.

There was a high incidence of both IgG and IgM antibodies in all the sera. The universal occurrence of higher level of humoral antibodies demonstrated in this study, suggested that most of the persons had been sensitised to the tubercle bacillus, though with a varying degree.

Jha et al (1974) studied 78 cases of active pulmonary tuberculosis and 25 healthy individuals as control and recorded IgG, IgM and IgA levels, quantitatively. The mean level of immunoglobulins was raised in pulmonary tuberculosis as compared to controls. There was no significant difference noted by the author viz-a-viz the extent of involvement of lung parenchyma. However, there was a tendency towards the increase in immunoglobulin level with the increasing severity of disease.

Lanzini et al (1976) studied 66 patients with bacteriologically or histologically confirmed tuberculous patients and established a spectrum of progressive human tuberculosis on clinical and immunological grounds. They classified tuberculous patients into four groups - (i) A polar reactive group (RR) with an active cell mediated immunity, with little or no antibody

response; (ii) A polar unreactive group (UU) characterised by rapid diffusion of lesion with poor or absent cell mediated immunity and marked antibody response; (iii) An intermediate reactive group (RI) characterised by good cell mediated immune response in vitro; (iv) An intermediate unreactive group with poor cellular reaction in vitro but these showed Jones-Mote skin reactions in vitro. (The number of tubercle bacilli in the tissues and the level of antibodies, both increased towards the unreactive end).

Skvor et al (1979) studied 46 pulmonary tuberculous patients with smear positive and a control group of 21 healthy subjects. The concentration of immunoglobulins was determined by single radial immunodiffusion technique. Tubercular patients were further evaluate (with regard to antibodies that reacted in haemagglutination reaction with tuberculo-proteins using human erythrocytes. Thus, values obtained were classified according to the extent of disease, which was closely related to the number of excreted bacilli. The results showed markedly elevated mean IgA level and significantly higher concentration of anti-old-tuberculin (O.T.) antibodies in the serum. The increase in

the average value of IgG and anti PPD antibodies was less pronounced. No correlation was found to exist between the higher titres of specific antibodies and IgA/IgG.

In 1981 Chatterji et al studied serum protein pattern in 35 tuberculous children under the age of 12 years and normal control cases, selected from the age group of 10 months to 12 years. They studied serum protein level by electrophoresis technique. Observation on serum protein pattern at the time of diagnosis, revealed definite alteration in different serum protein fractions. An early fall in albumin concentration and a rise in alpha-2 and gamma globulin fraction was the characteristic feature in majority of cases. Serial observations during the course of antitubercular treatment showed that good clinical improvement, in general, was associated with fall in alpha-2 globulin fraction and a rise in albumin component of the serum protein. Cases showing good clinical response to therapy also exhibited 5% or more rise in gamma globulin concentration over the intial value, after 4-6 weeks from the onset of therapy, followed by a gradual fall towards normal. Children who failed to show satisfactory response to

therapy did not actually exhibit the decrease in alpha-2 globulin concentration, opined the authors.

Although concentrations of total serum protein and different fraction reported by different workers vary to some extent, the normal values may be as follows (Chatterji et al 1981):

Total serum protein	-	6.3 - 8.2 gm%
Serum albumin	-	4.02 - 5.06 gm%
Gamma globulin	-	0.10 - 0.34 gm%
Alpha-2 globulin	-	0.30 - 0.93 gm%
Beta globulin	-	0.28 - 0.90 gm%
Gamma globulin	-	0.53 - 1.39 gm%

Daniel et al (1981) studied immune response to mycobacterial antigens in 65 patients with pulmonary tuberculosis, using delayed skin test reaction and ELISA also. Moreover, serum albumin and serum protein estimations were performed. Six out of 35 patients studied during the first month of therapy, had tuberculin skin test anergy. Author opined that failure to react to PPD was anergy and occured in patients with miliary tuberculosis, malnutrition and those having severe disease. The six anergic patients had some-what higher antibody titres to mycobacterial protein, but

not to polysaccharide antigens, as compared to non anergic patients.

Kardzito et al (1982) studied, the antibody level with the status of tuberculin reactivity and previous B.C.G. vaccination. The test was used to compare the level of type specific antibody IgG, IgM and IgA classes measured by ELISA in 107 patients with active pulmonary tuberculosis, 109 healthy tuberculin positive and 34 healthy tuberculin negative individuals. The authors observed that tuberculin positive control subjects had higher level of antibodies (IgG class only) than tuberculin negative control. In about 77% of the patients, measured antibody levels exceeded that seen in 97.5% of tuberculin negative control subjects. Only 62% of the patients had elevated antibody levels when compared to tuberculin positive controls. Previous B.C.G. vaccination had no significant effect on the antibody levels as observed by the authors.

Bhagonha et al (1983) studied immunological changes in pulmonary tuberculosis of 26 patients before the beginning of treatment and between third and fourth weeks of treatment. Out of 26 patients, 12 patients

were also investigated after four to six months of treatment. They measured immunoglobulin IgG, IgA & IgM by SIRD method; 'B' lymphocyte count by antisheep rabbit hemolysins and human complement; 'T' lymphocyte counts by sheep red cells. They used PHA and PPD for morphological evaluation of the lymphoblastic transformation. Normal levels of immunoglobulin were found in tubercular patients before the treatment. However, they noted that patients treated without rifampicin showed a gradual and significant increase in IgG and decrease of IgM with respect to the initial values. The IgA, despite the fact that this also decreased, did not do so significantly. The patient, whose therapy included rifampicin, did not present any significant difference with regard to the initial value and the value after four months of treatment; although the IgA level was significantly higher between the fourth and sixth months of treatment.

Grange et al (1984) studied acute phase reactant proteins and immunoglobulins in sera from 107 smear positive pulmonary tuberculosis patients and 144 healthy subjects, by laser nephelometery. These levels were correlated with clinical, haematological

and radiological features and the diameters of the tuberculin skin test read at various times. Authors observed that levels of all acute phase reactants increased significantly in tuberculosis except for that of transferin, which was lowered. There was significant correlation between the level of some of the acute phase reactants and antibodies to M. tuberculosis viz - IgG, IgA and IgM in that order of magnitude. Serum protein levels were not correlated with the extent of disease but had better correlation with ESR and leucocyte count. Transferrin levels tended to be higher in those with chronic disease. Among the haematological findings, the most significant observation was the negative correlation between lymphocyte count and haptoglobin level, suggesting a possible regulatory role for this protein.

Singh et al (1984) investigated 30 untreated sputum positive cases of pulmonary tuberculosis. Serum levels of IgA, IgM and IgG were estimated by single radial immunodiffusion method. Findings were compared to 20 healthy control subjects. Serum levels were also estimated at 3, 6 and 9 months interval, after starting

the treatment. Authors observed that before starting the treatment mean level of each of the three immunoglobulin classes was higher in case of pulmonary tuberculosis as compared to control. After the treatment, immunoglobulin levels, especially of IgG and IgA declined in patients who improved, while patients who did not improve, the levels tended to rise while concluding their findings authors remarked that there was a definite polyclonal, humoral response to pulmonary tuberculosis in the form of significantly raised level of serum immunoglobulins. Moreover, there was an antigenic over load present in the body, as evidenced by the fact that a persistently high level of immunoglobulins was seen in patients who did not improve whereas a lower level was found in patients who improved with chemotherapy.

Seth et al (1985) studied 60 children of pulmonary primary complex (PPC) in which 15 were symptomatic and mantoux positive (SMP) cases and 20 were age matched controls of equivalent nutritional status. Immunoglobulin levels were estimated by SRID method. Nutritional assessment was done by weight for age criteria. Serum protein and its fractions viz albumin,

globulin and IgG levels were significantly increased whereas IgM levels were significantly decreased in children with PPC and SMP in comparison to control cases. IgA levels were comparable in both groups. The study suggested that Pulmonary Primary Complex (PPC) & Symptomatic-Mantoux Positive (SMP) were the milder manifestation of tuberculosis in children, each having adequate humoral immunity. Thus, the author observed that these two clinical forms of tuberculosis do not confirm to the immune spectrum of tuberculosis in adults as described by Lenzini et al (1977).

Seth & Singh (1987) in their study of humoral immunity in progressive primary disease (PPD) and tubercular meningitis (TBM) estimated immunoglobulins, total and differential proteins. The IgG level was significantly less in TBM as compared to controls. The reduction was even more significant when comparison was made with PPC and PPD groups. This was due to a significant rise in the level of IgG in these two categories as compared to control cases. The level of IgM was lower in all the diseased groups and it was significantly reduced in TBM cases.

Okpapi et al (1989) studied alpha-1-antitrypsin, immunoglobulins and different radiological type of pulmonary tuberculosis in Nigerians. The authors observed that tuberculin skin test tended to be more intensively reactive in caseo-nodular and other groups than in miliary group. Serum IgA, IgM and alpha-1-antitrypsin levels were higher in Nothern Nigerians with pulmonary tuberculosis than in controls. IgA and IgG levels were higher in miliary tuberculosis than in caseonodular and cavitating tuberculosis.

Greinert et al (1989) investigated immunologic parameters in patients with lung tuberculosis, at various stages, with special reference to serum IgE concentration. The author observed that delayed type of hypersensitivity reaction was weakened in patients with lung tuberculosis, depending on the severity of disease. The same type of results was observed in cases of non miliary tuberculosis. On analysing the mononuclear cells of peripheral blood, abnormalities were found and in particular, an increase in the monocyte count was observed. In addition to an increase in the level of serum immunoglobulin A and G, circulating immune complexes were detected in 50% of the patients. In 50% of the

patients, authors found elevated IgE serum levels, which decreased in response to treatment but did not return to normal.

Greinert et al (1990) observed increased serum IgE concentration in about one half of patients with pulmonary tuberculosis. There was a tendency for IgE level to regress in patients who were under anti-tubercular treatment.

Kumar (1990) studied 26 children suffering from primary complex, 9 cases of tubercular basal meningitis (TBM) in the age group of 5 to 12 years. He took 16 normal children as age matched controls. He found a rise in serum level of immunoglobulins in primary complex cases who were mantoux negative, mantoux positive and BCG vaccinated. Rise in the immunoglobulin was statistically significant in the vaccinated group. The IgG levels were decreased in cases suffering from TBM and miliary tuberculosis, indicating severity of the disease.

Wong et al (1990) studied changes in the serum protein (albumin), immunoglobulins and acute phase reactants in pulmonary tuberculosis, during therapy.

The serum level of immunoglobulins, albumin and acute phase reactants were measured by immuno-electrophoresis in 57 patients with pulmonary tuberculosis. They were first diagnosed and then followed at one month, 2 months and 4 months intervals during antitubercular treatment. These values were compared to those of 41 healthy controls. Significant increase in serum IgG, IgM, alpha-2-antitrypsin and haptoglobulin levels were observed by the authors, while the level of transferrin and alpha-2-macroglobulin were significantly reduced. No changes were observed in the levels of serum albumin, IgA and complement C3. However, after four months of treatment IgG and alpha-1-antitrypsin levels were still significantly elevated and that of haptoglobulin remained significantly lower compared to control values.

Murthy et al (1991) studied serum and CSF immuno-globulins in 50 cases of meningitis. Total proteins and immunoglobulins were estimated by single radial immuno-diffusion technique. In this study authors observed that serum and CSF, IgG, IgA and IgM were significantly raised in patients of TBM, when compared with age and sex matched

controls. The mean value of CSF, IgG expressed as percentage of total CSF proteins was reported to be of high diagnostic significance to differentiate between TBM and pyogenic meningitis.

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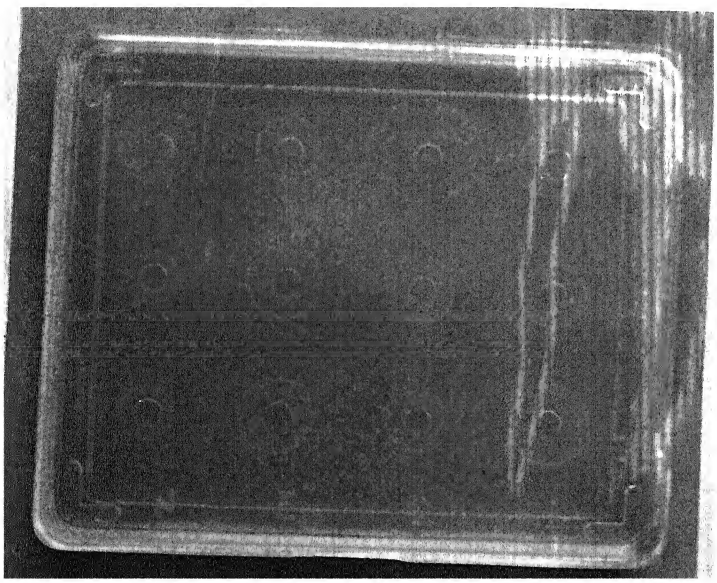

MATERIAL & METHODS

MATERIAL AND METHODS

The present study was carried out in the department of Paediatrics, M.L.B. Medical College, Jhansi. The clinical material of this study comprised of 26 children attending the Out Patient Department and those admitted in children's ward.

The selection of cases was based on clinical symptomatology (fever, cough, weight loss, anorexia and failure to thrive). Mantoux test was performed in each case. At the same time, 6 healthy children, matched for age, sex and nutritional status, served as control cases and were subjected to the same investigations as the study group.

Type of cases :

The following types of tuberculous cases were taken for this study.

1. Primary pulmonary complex (PPC)
2. Progressive pulmonary disease (PPD)
3. Tubercular basal meningitis (TBM)

A detailed clinical history (present and past illness), family history including socio-economic status, history of contact were taken in all the children.

The immunisation status of each child was assessed. The nutritional status assessment was done by weight for age criteria. A thorough clinical examination was done in each case (as per proforma).

Following investigations were done in each cases :

(A) Haemogram :

1. Haemoglobin :

Hb% estimation was done by Sahli's method based on quantity of blood converted into hematin with diluted hydrochloric acid.

2. Total Leukocyte Count (TLC) :

This was done by using Neubaur's chamber.

3. Differential Leukocyte Count (DLC) :

A thin and uniformly prepared peripheral smear was stained by Leishman stain. Leukocyte count was done by using oil immersion and percent distribution of different leukocyte was obtained by counting 200 cells.

4. Estimation of ESR :

This was done by Wintrobe method. A haematocrit tube was filled, upto the 100th mark, with oxalated blood and allowed to stand vertically for an hour. Thereafter, reading was taken.

(B) Tuberculin test :

Intradermal injection of 0.1 ml of 5 tuberculin unit of PPD-RT-23, with tween 80 as stablizer was injected on the volar surface of forearm. The result was read after 72 hours after injection. Size of 10 mm or more was taken as positive.

(C) Chest Skiagram :

Posterior-anterior view was taken.

(D) Estimation of total serum proteins and fractions-(albumin and globulin) :

Quantitative estimation of total proteins and albumin were done by BIURET and Bromocresol green (BCG) methods respectively.

Principle :

Total proteins estimation is based on the classical Biuret reaction. The peptide bonds present in polypeptide molecular complex bind cupric ions in alkaline medium, to give a violet colour. The violet colour, so developed, is proportional to total protein concentration which is measured photometrically at 546 nm (530 to 570 nm) or with green filter.

The dye Bromocresol green (BCG) in a buffered acid medium selectively binds albumin and forms a complex. During reaction, the initial yellow colour quantitatively changes to green. The green colour, so developed is proportional to albumin concentration and is measured photometrically at 628 nm (600 to 650 nm) or with Red filter.

Procedure :

Three test tubes were marked as Blank (B), Standard (S) and Test (T) respectively. Blank test tube contained distilled water while 'S' and 'T' test tubes contained standard solution of human proteins and test serum respectively.

Total proteins :

For 3 ml covet, 3 ml Biuret reagent and 0.05 ml distilled water/standard/sample were taken in the labelled test tubes respectively. The contents in each test tube were mixed and all three tubes were allowed to stand at room temperature for 20 minutes. The absorbance of test, standard and blank were read at 546 nm wave-length.

Albumin :

For 3 ml covet, 3 ml bromocresol green (BCG) reagent and 0.02 ml each of distilled water/standard/ sample were used in the labelled test tubes respectively. The contents in test tubes were mixed and absorbance of test, standard and blank read after one minute at 628 nm wave length.

CALCULATION :

Total proteins concentration (gm/dL) =

$$= \frac{\text{Optical density (OD) of test sample}}{\text{Optical density (OD) of standard}} \times \text{Total protein concentration of standard.}$$

Albumin concentration (gm/dL) =

$$= \frac{\text{OD of test sample}}{\text{OD of standard}} \times \text{Albumin concentration of standard .}$$

The value of globulin was calculated by difference of total proteins and albumin.

(E) CSF Examination - This was done whenever required.

(F) Estimation of serum immunoglobulins :

Quantitative estimation of immunoglobulin - IgA, IgM and IgG was done by single - Radial Immunodiffusion (SRID) method. (Mancini et al 1965).

Principle :

The method is based on precipitation of reactants present in the serum and in the gel. The gel contains monospecific antibody to the component of serum being quantitated.

The concentration of antibody is so adjusted as to be less than the concentration of component of serum being quantitated : the latter diffuses out in a radial fashion from the well. The diameter square of ring, so formed, is proportional, to the concentration of component.

Procedure :

Commercially available agarose gel plates incorporating monospecific antibodies to either IgA, IgM or IgG were obtained for estimation of immunoglobulins. Moreover, standard serum containing -

IgA	1.4 mg/ml	99 IU/ml
IgM	1.10mg/ml	130 IU/ml
IgG	9.00mg/ml	112 IU/ml

was obtained from commercial sources.

Three dilutions : 75%, 50% and 25% of the standard serum were prepared. 5 ul of each of these dilutions as well as 5 ul of undiluted standard sample was poured into the wells of agarose gel plate containing antibody. Serum sample from patients which had been preserved at - 20°C were thawed on the day of the test. The dilutions were made according to instructions provided by the manufacturer for each immunoglobulin test plate. 5 ul of each serum sample was poured into one specific well on each of the three plates. The plates were incubated in inverted position at room temperature for 72 hours.

Recording of Results :

Diameter of each precipitin ring was measured by a scale, nearest to 0.1 mm and then converted to diameter square.

Reference curve for each test was drawn after plotting the diameter square on X-axis against known concentrations of reference serum plotted on Y-axis. Reading of unknown (from patient's precipitin ring diameter square) was read from the reference curve.

Final results were obtained after taking into account dilution factor, if used.

OBSERVATIONS

OBSERVATIONS

This study was conducted on 26 children attending the Paediatric department of M.L.B. Medical College Hospital, Jhansi. Six healthy, age and sex matched children were also studied and subjected to the same investigations as the study group. This control group formed the basis for comparison of results obtained in the study group.

Age and Sex distribution :

Table - I shows that among study group, there were 15 male and 11 female children. The maximum number of cases (46.15%) were seen in the age group 3-6 years followed by 30.76% in the age group of 6-9 years. The control group consisted of six healthy children in the age group of 3-12 years with almost similar pattern of age distribution. The male and female ratio, in control and study group was 1:1 and 1.36:1 respectively.

TABLE - I : Age and sex distribution of study and control groups.

Age group (Yrs)	Study group			Control group		
	Male	Female	Total	Male	Female	Total
3-6	5	7	12	1	1	2
6-9	6	2	08	1	1	2
9-12	4	2	06	1	1	2
Total	15	11	26	3	3	6

2. Type of cases in the study group :

Table - II depicts the type of tuberculars cases who were selected i.e. Primary Pulmonary Complex (PPC), Progressive Primary Disease (PPD) and Tubercular basal Meningitis (TBM). Out of 26 cases, 13 cases of PPC (50%), 8 cases of PPD (30.76%) and 5 cases of TBM were selected for study.

TABLE - II : Type of cases in study group

Sl. No.	Clinical types	No. of cases		
		Male	Female	Total
1.	PPC	6	7	13
2.	PPD	6	2	08
3.	TBM	3	2	05
	Total	15	11	26

3. Nutritional status of children in the study and control groups :

Table - III depicts that maximum number of cases were seen in grade I and II malnutrition in the study and control group. There were 3 cases of the study group falling in grade - III malnutrition, 2 had TBM and 1 case had PPC. There was no control case having grade - III malnutrition.

TABLE - III : Nutritional status of children in study and control group

Sl. No.	Nutritional grade	Study group				Control group	Total
		PPC	PPD	TBM	Total		
1.	Normal grade	1 (7.64%)	2 (25%)	1 (20%)	4	2 (33.33%)	2
2.	Grade I & II	11 (84.6%)	6 (75%)	2 (40%)	19	4 (66.66%)	4
3.	PEM (Grade-III)	1 (7.64%)	-	2 (40%)	3	-	-
	Total	13	8	5	26	6	6

4. Status of Mantoux test :

Table - IV shows that mantoux test was positive in 77% of cases of the study group, while all the cases of control group and those of TBM in the study group showed negative mantoux test. The PPC and PPD cases showed 100% and 98% mantoux positivity respectively.

TABLE - IV : Status of Mantoux test in study and control groups

Sl. No.	Size of induration (diameter in mm)	Study group				Control group	Total
		PPC	PPD	TBM	Total		
1.	0-10	-	1	5	6	5	5
2.	10-20	9	4	-	13	-	-
3.	20-30	4	3	-	7	-	-
	Total	13	8	5	26	5	5

5. Haemogram in study and control group :

Table - V depicts the haemogram in control and study groups which included different types of tuberculosis. The haemoglobin level showed a decline from patients with pulmonary form of tuberculosis to those with TBM. The ESR was raised significantly in all groups of cases as compared to control. The lymphocytosis was more common in TBM as compared to control cases.

TABLE - V : Haemogram in control and study groups

Sl. No.	Clinical types	Hb% mean ± SD (Gm%)	TLC mean ± SD (per Cuml)	Lympho-cytes mean±SD (%)	ESR mean ± SD (mm)
I.	CONTROL (n = 6)	11.83 ± 1.03	8133.0 ± 446.0	41.8 ± 2.5	18.6 ± 4.8
II.	STUDY -				
	(A) PPC (n=13)	10.93 ± 1.20	8807.0 ±1009.5	48.15 ± 9.47	39.0 ± 9.6
	(B) PPD (n=8)	11.03 ± 0.42	8637.5 ± 604.5	47.62 ± 5.80	41.07 ±13.50
	(C) TBM (n=5)	9.28 ± 1.43	9340.0 ± 118.59	55.6 ± 9.3	45.14 ± 8.32

6. Total plasma proteins and fractions in PPC group :

Table-VI depicts total plasma proteins and fractions in PPC and control group. The mean total plasma proteins level in PPC as a whole was 7.07 ± 0.81 gm/dL while 7.4 ± 0.6 gm/dL in control group.

Serum albumin level in PPC and control group was 4.26 ± 0.62 gm/dL and 4.71 ± 0.41 gm/dL respectively.

Serum globulin level in PPC and control group was 2.89 ± 0.41 gm/dL and 2.56 ± 0.32 respectively.

On comparison of total protein and its fraction in PPC with control group, there was no significant difference observed on application of 't' test.

TABLE - VI : Total plasma proteins and fraction in PPC and control group

Sl. No.	Test	PPC (n = 13) mean ± SD	Control (n = 6) mean ± SD	't' value	P value
1.	Total proteins	7.07 ± 0.81	7.4 ± 0.6	0.89	$\nearrow$0.1
2.	Serum Albumin	4.26 ± 0.62	4.71 ± 0.41	1.50	$\nearrow$0.1
3.	Serum globulin	2.89 ± 0.41	2.56 ± 0.32	1.73	$\nearrow$0.1

P $\nearrow$0.1 - Not significant.

7. Total Plasma Proteins and fractions in PPD and control group :

Table - VII shows total plasma proteins and fractions in PPD and control group. On observing the table, the mean level of total plasma protein in study and control groups was 7.05 ± 1.09 mg/dl and 7.4 ± 0.6 gm/dl respectively.

Serum albumin level in PPD and control group was 4.2 ± 0.57 and 4.71 ± 0.41 gm/dl respectively.

Serum globulin level in PPD and control group was 2.87 ± 0.36 and 2.56 ± 0.32 gm/dl respectively.

There was no significant difference of plasma protein and its fractions in PPD and control group on application of 't' test.

TABLE - VII : Total plasma proteins and fraction in PPD and control cases.

Sl. No.	Test	PPD (n = 8) mean ± SD (gm/dl)	Control (n = 6) mean ± SD (gm/dl)	't' value	'p' value
1.	Total plasma protein	7.05 ± 1.09	7.4 ± 0.6	0.92	>0.10
2.	Serum albumin	4.2 ± 0.57	4.71± 0.41	1.7	>0.1
3.	Serum globulin	2.87 ± 0.36	2.56± 0.32	1.55	>0.10

P >0.1 - Not significant.

8. Total Plasma Proteins and fractions in TBM and control cases :

Table - VIII depicts total plasma protein and its fractions in TBM and control group. The mean level of total plasma protein in TBM and control cases was 7.04 ± 1.23 mg/dl and 7.4 ± 0.6 gm/dl respectively. Thus, no significant difference observed between two groups.

The mean level of albumin in TBM and control cases was 4.16 ± 0.89 and 4.71 ± 0.41 gm/dl respectively. There was a slightly lower level of serum albumin in TBM as compared to control cases.

The mean globulin level in TBM and control cases was 2.92 ± 0.36 gm/dl and 2.56 ± 0.32 gm/dl respectively. There was no significant difference between two groups.

On application of 't' test, there was no significant statistically while comparing TBM with control cases.

TABLE - VIII : Total serum proteins and fractions in TBM and control cases.

Sl. No.	Test	TBM (n = 5) mean ± SD (gm/dl)	Control (n = 6) mean ± SD (gm/dl)	't' value	'p' value
1.	Total plasma proteins	7.04 ± 1.23	7.4 ± 0.6	0.78	>0.1
2.	Serum Albumin	4.16 ± 0.89	4.71± 0.41	0.76	>0.1
3.	Serum globulin	2.92 ± 0.36	2.56 ± 0.32	1.6	>0.1

P >0.1 - Not significant.

9. Serum Immunoglobulins in PPC and control groups :

Table - IX shows immunoglobulins level in PPC and control groups.

The mean level of IgA was 129.76 ± 51.22 IU in PPC cases whereas in control cases, level was 101.97 ± 38.47 IU. Thus, IgA level was raised in PPC cases as compared to control.

The mean level of IgM in PPC (179.96 ± 53.0) was lower when compared to control cases (231.0 ± 57.77 IU).

The mean level of IgG in PPC and control cases was 197.0 ± 65.22 IU and 141.36 ± 27.66 IU respectively.

There was a slight increase in IgG level as compared to control group.

But, statistically mean levels of IgA, IgM and IgG groups was not significantly different when compared with control cases, following 't' test.

TABLE - IX : Serum immunoglobulins in PPC and control group :

Sl. No.	Test	PPC mean ± SD IU	Control mean ± SD IU	't' Value	'p' value
1.	IgA	129.76 ± 51.22	101.97±38.47	1.16	7 0.1
2.	IgM	179.96 ± 53.00	231.00±57.77	1.80	7.05
3.	IgG	197.00 ± 65.22	141.36±27.66	1.70	7.05

IgA - 7 0.1 Not significant
IgM - 7.05 Not significant
IgG - 7.05 Not significant

10. Serum Immunoglobulins in PPD cases :

Table X depicts serum immunoglobulins in PPD and control cases.

The mean level of IgA was highest in PPD (133.85 ± 48.0 IU) as compared to control cases (101.97 ± 38.47 IU).

The IgM level in PPD (166.07 ± 60 IU) was lower when compared with control cases (231.0 ± 57.77 IU). The mean level of IgG in PPD and control cases was (189.05 ± 51.48 IU) and 141.36 ± 27.66 IU respectively. There was a slightly higher level of IgG in PPD as compared to control cases.

Although IgG and IgA levels were higher in PPD cases in comparison to control cases, statistically these had no significance on application of 't' test.

TABLE - X : Serum Immunoglobulins in PPD and control cases :

Sl. No.	Test	PPD Mean ± SD IU	Control Mean ± SD IU	't' value	'p' value
1.	IgA	138.85 ± 48.0	101.97 ± 38.47	1.46	>0.1
2.	IgM	166.07 ± 60.0	231.0 ± 57.77	1.89	$>.05$
3.	IgG	189.05 ± 51.48	141.36 ± 27.66	1.83	$>.05$

IgA p >0.1 - Not significant
IgM p $>.05$ - Not significant
IgG p $>.05$ - Not significant

11. Serum Immunoglobulins in TBM cases :

Table XI shows serum immunoglobulins in TBM cases.

The mean level of IgA in TBM (71.08 ± 15.96) was lower as compared to control cases (101.97 ± 38.47 IU).

The IgM level in TBM (128.7 ± 32.18 IU) was lower in comparison to the control group.

The IgG level (93.22 ± 20.2 IU) in TBM was also reduced when compared to control cases (141.36 ± 27.66 IU).

Thus IgA, IgM and IgG levels were reduced in TBM cases in comparison to control cases.

Statistically, the decreased level of IgG and reduced level of IgM in TBM cases, as compared to control cases, was significant. IgA level was also reduced substantially but this was not significant on application of 't' test of significance.

TABLE - XI : Serum immunoglobulins in TBM and control cases :

Sl. No.	Test	TBM Mean ± SD (IU)	Control Mean ± SD(IU)	't' value	'p' value	
1.	IgA	71.08 ± 15.96	101.97 ± 38.47	1.10	>0.1	
2.	IgM	128.70 ± 32.18	231.00 ± 57.77	2.52	<0.05	**
3.	IgG	93.22 ± 20.02	141.36 ± 27.66	2.30	<0.05	**

IgA >0.1 - Not significant
IgM <0.05 - ** Significant
IgG <0.05 - ** Significant

12. Correlation of serum immunoglobulins in PPC and TBM :

Table XII shows correlation of different immunoglobulins in between PPC and TBM cases.

All the immunoglobulins levels were lower in TBM when compared with PPC cases. This reduction of IgG and IgA was more when compared with control, since levels of immunoglobulins in PPC were already raised.

On application of 't' test of significance between PPC and control TBM cases, the decreased level of IgG and IgA was statistically significant. IgM level was also decreased but the reduction was not significant statistically.

TABLE - XII : Correlation of serum immunoglobulins in PPC & TBM

Sl. No.	Test	PPC mean ± SD(IU)	TBM mean ± SD(IU)	't' value	'p' value
1.	IgA	129.76 ± 51.22	71.08 ± 15.96	2.23	$<$0.05 **
2.	IgM	179.96 ± 53.00	128.70 ± 32.18	1.84	$>$.05
3.	IgG	197.00 ± 65.22	93.22 ± 20.22	2.68	$>$.01 **

IgA $<$ .05 ** Significant
IgM $>$ .05 Not significant
IgG $>$ .01 ** Significant

13. Correlation of immunoglobulins between PPD and TBM cases :

Table XIII depicts immunoglobulins in PPD and TBM cases.

The IgA level was highly decreased in TBM (71.08 ± 15.96 IU) as compared to PPD (138.85 ± 48.0 IU). The IgM level was slightly decreased in TBM (128.7 ± 32.18 IU) when compared with PPD cases (166.07 ± 60.0 IU). The mean level of IgG in TBM (93.22 ± 20.02 IU) was highly reduced as compared to PPD (189.05 ± 51.48 IU).

Thus, all the level of immunoglobulins were reduced in TBM cases when compared with PPD cases.

The reduction of IgG and IgA level was statistically significant.

TABLE - XIII : Correlation of Immunoglobulins between PPD and TBM

Sl. No.	Test	PPD mean ± SD(IU)	TBM mean ± SD(IU)	't' value	'p' value	
1.	IgA	138.85 ± 48.00	71.08 ± 15.96	2.3	< 0.05	*
2.	IgM	166.07 ± 60.00	128.70 ± 32.18	1.23	> 0.1	
3.	IgG	189.05 ± 51.48	93.22 ± 20.02	2.64	< 0.05	**

IgA < 0.05 * Significant
IgM > 0.1 Not significant
IgG < 0.05 ** Significant

14. Correlation of immunoglobulins between PPC and PPD cases :

Table XIV depicts immunoglobulins level of PPC and PPD cases.

On observing the table it is evident that there were no significant difference between mean levels of immunoglobulins of two groups of cases.

TABLE - XIV : Correlation of immunoglobulins between PPC and PPD

Sl. No.	Test	PPC mean ± SD(IU)	PPD mean ± SD(IU)	't' value	'p' value
1.	IgA	129.76 ± 51.22	138.85 ± 48.00	0.40	$\overline{/}$0.5
2.	IgM	179.96 ± 53.00	166.07 ± 60.00	0.56	$\overline{/}$0.5
3.	IgG	197.00 ± 65.22	189.05 ± 51.48	0.29	$\overline{/}$0.5

P value $\overline{/}$0.5, not significant.

DISCUSSION

DISCUSSION

Tuberculosis forms a major health problem in India, despite all microbiological advancement. Diagnosis of an active case of tuberculosis is still far from satisfactory.

The detection of Koch's bacillus may be more difficult in childhood, even for specialized laboratories. It has been reported that if bacilli in the sputum of patient of pulmonary tuberculosis is less than 50,000/ml laboratory may report the sputum as negative. Similarly culture has its own deficiencies and radiology is often atypical. Clinical examination may be unproductive. So the diagnosis is often largely based on tuberculin test.

The present study was designed to investigate humoral response (IgA, IgM, IgG) in various form of childhood tuberculosis viz primary pulmonary complex (PPC), progressive primary disease (PPD) and tubercular basal meningitis (TBM). Nutritional status of children was assessed by weight for age criterion of Nutritional subcommittee of Indian Academy of Paediatrics.

The present study comprised of 26 children suffering from tuberculosis. Six healthy age and sex matched children served as the control group in the present study.

The maximum number of cases were seen in the age group of 3-6 yrs (46.15%) followed 30.76% in the age group of 6-9 years and 23.09% cases was found in 9-12 years age group.

The male and female ratio in control and study group was 1:1 and 1.36:1 respectively. There was slightly predominances of male over females in the study group. This is obious that males are better cared than females.

The selection of cases was based on clinical symptomatology viz fever, cough, weight loss, anorexia, and failure to thrive. Mantoux test was performed in each case. Healthy children were also subjected to same investigations as study group.

Total plasma proteins and differential fractions were studied in each group of cases. Immunoglobulin (IgA, IgM and IgG) levels were measured by the single radial immunodiffusion (SRID) method of Mancini et al (1965) in each and every cases.

In this study, three types of tuberculous cases were selected for the study viz primary pulmonary complex (PPC), progressive primary disease (PPD) and tubercular basal meningitis (TBM). Out of 26 cases 50%

cases belonged to PPC followed by 30.76% cases of PPD and 19.24% cases of TBM was taken in the study group. Clinically, the PPC was the commonest and mildest form of tuberculosis whereas, TBM was of the severest form of tuberculosis, PPD being of intermediate severity.

The objective of the present study was to know the nonspecific humoral immune response for the application of serodiagnostic method in these types of tuberculosis having varying severity.

The nutritional status of children in the study and control group was studied by weight for age criterion laid by Nutritional subcommittee of Indian Academy of Paediatrics. The maximum number of cases were seen in grade I and grade II malnutrition in the study and control group (84.6% and 66.66% respectively). Out of three cases of study group falling in grade III malnutrition, two had TBM and one case had PPC. There was no control case having grade III malnutrition. However, total and differential serum proteins were comparable in PPC, PPD and TBM cases.

Decreasing levels of haemoglobin were noted from control group to pulmonary tuberculosis cases and children with TBM.

On an average, patients with pulmonary tuberculosis had Hb% level of 10.93 ± 1.2 mg%. Compared to this, TBM cases had mean Hb% level of 9.28 ± 1.43 gm%. ESR was raised significantly in all the clinical groups of patients, in comparison to control cases. Lymphocytosis was more common in TBM as compared to control cases.

Similar results have been obtained by Kardjito and Grange (1980). They have explained that the elevation of white cell count in disease was due to a significant increase in neutrophils and monocytes. Grange et al (1984) have found a significant association between the extent of disease and ESR. They opined that the best indicators of the extent of disease, apart from radiology, appeared to be the ESR and leukocyte count.

Serum Proteins and fractions in tuberculosis :

The distribution of serum proteins in tuberculosis in human being has been a common subject of intensive study. It has been established that in active infection (even a milder form), such as that which follows BCG administration, significant changes in serum proteins occur (Gilliland et al 1958). The alteration in serum proteins can be caused by inadequate protein intake due

to illness, insufficient synthesis or degree of tissue distruction owing to activity of disease. The general trend being a decrease in the albumin concentration and increase in globulin concentration.

Total Plasma Proteins : (Table no. VI, VII, VIII)

In the present study, mean level of total plasma protein in PPC was 7.07 ± 0.81 gm/dl whereas the mean level in control group of cases has 7.4 ± 0.6 gm/dl. The mean level of total plasma proteins in PPD and TBM cases was 7.05 ± 1.09 gm/dl and 7.04±1.23 mg/dl respectively. On statistical analysis, it was seen that there was no difference in the total plasma proteins in the various groups of tuberculosis viz PPC, PPD and TBM amongst themselves as well as when compared to the values observed in control group of cases ($p > 0.1$).

Similar results were also reported by different workers (Rao 1974, Chatterjee et al 1981, Seth et al 1985).

Chatterjee et al (1981) noted average values of total protein in different types of tuberculosis viz simple primary, progressive primary and in cases complicated with meningitis. The values were found to be 7.2 gm/dl 7.1 gm/dl and 7.8 gm/dl respectively in various form of tuberculosis.

Seth et al (1985) in their study of 50 children with primary pulmonary complex (PPC), 15 cases with symptomatic mantoux positivity (SMP) and 20 healthy age matched control reported the values of serum protein to be 7.52 $\pm$ 1.17 gm/dl, 7.78 $\pm$ 0.74 gm/dl and 7.40 $\pm$ 1.31 gm/dl respectively. They also like us reported no significant difference in total proteins in the three groups.

Nemir et al (1961) did not put any importance on total protein, but on percentage changes in different serum fractions in tuberculosis in childfen. Udani et al (1964) noted slight increase of total protein in tuberculous meningitis cases.

The finding of progressive decrease in total protein values in pulmonary tuberculosis from minimal to far advanced cases was observed by Bovornkitti (1961). He explained his finding by inadequate protein intake of chronically ill patients and by insufficient albumin synthesis in advanced stage of disease because of impairement in liver function.

Maintenance of fairly normal total protein values in spite of some increases in globulin fraction has been explained by regulative mechanism which attempts to maintain the colloid osmotic pressure by increasing the plasma volume (quoted by Bovornkitti et al 1961).

Serum albumin : (Table VI, VII and VIII)

In the present study mean level of albumin in PPC, PPD and TBM cases was 4.26 gm/dl, 4.2 ± 0.5 gm/dl and 4.16 ± 0.83 gm/dl respectively.

The mean level of albumin in control group was 4.71 ± 0.41 gm/dl, which was statistically in-significant for the values observed in all the three groups ($p > 0.1$). A significant finding was a decreasing trend in serum albumin levels in various form of tuberculosis viz PPC, PPD and TBM cases. However, this decreasing trend was not significant statistically while comparing with control group. The decrease in albumin level may be explained by poor intake and impairement of liver synthesis of albumin as the severity of disease progressed. But serum albumin value was comparable in PPC, PPD and TBM cases.

Similar findings have been observed by various other workers (Seibert et al 1942, Nemir et al 1961, Chatterjee et al 1981, Rao et al 1974).

Seibert et al (1942) noted that reduction in albumin though present in all types of tuberculosis, was more marked in moderate to severe form of disease.

Rao et al (1974) reported similar findings in their study of children between 1 and 15 years. They found no significant changes in albumin fraction of simple primary leisons, though the values were in lower limit of normal in PPD cases. However, moderate decrease in serum albumin was found in TBM cases.

Chatterjee et al (1981) also reported similar trend in serum albumin level in various form of tuberculosis.

Serum Globulin : (Table VI, VII and VIII)

In the present study, serum globulin level in PPC, PPD and TBM was 2.89 $\pm$ 0.41 gm/dl, 2.87 $\pm$ 0.32 gm/dl and 2.92 $\pm$ 0.36 gm/dl respectively. The mean level of serum globulin in control group was 2.56 $\pm$ 0.32 gm/dl.

Although these findings revealed that the average value of globulin was increased in PPC, PPD and TBM however, no statistical significant difference in serum globulin value was observed when compared to the values in control group. Further, it was seen that serum blobulin values was comparable with each other in various form of tuberculosis in this study. Only Seth et al (1985) have reported total serum globulin values in symptomatic mantoux positivity (SMP) and primary pulmonary complex (PPC) cases. They found no significant difference in serum globulin values in those groups when compared to

However, various other workers reported changes in various fractions of globulin on electrophoretic pattern α-2 globulin, β - globulin and γ-globulin values were elevated in various form of tuberculosis (Rao et al 1974, Chatterjee et al 1981).

Serum Immunoglobulins in tuberculosis :

It hasbeen established by various workers that there is a definite antibody response to tuberculous disease in human beings and levels of all the three immunoglobulin classes viz IgG, IgA and IgM are significantly altered as compared to the matched control cases.

Immunoglobulin A : (Table IX, X and XI)

In this study, mean level of IgA in PPC, PPD and TBM was 129.76 $\pm$ 51.22 IU, 138.85 $\pm$ 48.00 IU and 71.08 $\pm$ 15.96 IU respectively. The value of IgA in control group was 101.97 $\pm$ 38.47 IU.

It was evident that the mean level of IgA was increased in PPC and PPD cases as compared to control cases. However, IgA level in TBM was lower than the control values. Statistically no significant difference in IgA values was observed when individual groups of patients were compared to the control.

The finding of this study was in agreement with the finding of various workers (Faulkner et al 1967, Sergovia and Fishbein 1971, Jha et al 1974, Skvor et al 1979, Agnihotri et al 1978, Singh et al 1984, Okpai et al 1989, Kumar 1990).

The reason for a rise in IgA level is not clear. It is, however, likely that the lung, which is the site of tuberculous leisons (known to contain a high population of IgA producing immune cells) may be responsible (South et al 1967). This rise in IgA level is especially important in local immune response to a low grade infection.

Singh et al (1984) opined that significantly higher level of IgA in pulmonary tuberculosis was an indication of definite humoral response. They said that the increase of IgA was probably a reflection of an increase in the surface antibody in the lung secretions.

Okapai et al (1989) reported higher level of IgA in various Nigerians with pulmonary tuberculosis, than control group.

However, Seth et al (1985) reported that IgA level in primary pulmonary complex and symptomatic mantoux positivity (SMP) cases was comparable to control.

Seth et al (1987) found significant decreased level of Ig^A in TBM cases while Ig^A level in PPC and PPD cases were comparable to control group. Wong et al (1990) found no change in Ig^A level in comparison with control cases.

Immunoglobulin M : (Table IX, X, XII)

In the present study, the IgM level in PPC, PPD and TBM was 179.96 ± 53.0 IU, 166.07 ± 60.0 and 128.7 ± 32.18 IU respectively, while the IgM level in control group was 231.0 ± 57.77 IU.

It was evident that IgM level was decreased in all the groups of tuberculosis viz PPC, PPD and TBM, when compared to the values observed in the control group. Although amongst the three groups, the values were only statistically significant with the TBM group.

The low level of IgM in the present study may explained on the basis of :

(i) Existence of IgM antibodies for a short time after initiation of immune response.

(ii) Tuberculosis being a chronic disease, it is possible that IgM antibody disappear early.

(iii) Adjuvant effect of mycobacteria results in a significant increase in total serum IgG and IgA but not in IgM and;

(iv) Contact with mycobacterial antigens naturally or by BCG vaccination results in secondary type humoral immune response in active tuberculosis without high IgM concentration. However, in the kinetics of humoral immune response in tuberculosis it remains to be shown whether IgM antibody against PPD are elevated in the earlier phases of tuberculosis, so as to determine whether their demonstration can be useful in the early diagnosis of disease (Viljanen 1982, quoted by Seth et al 1987).

The decreased level ofIgM may be explained on the basis that in primary bacterial infection, antibody of IgM class becomes available for opsonization, early in the course of illness and being confined to the blood, are consumed in case of bacterimia when the disease has already progressed out of the phase of the early intervention of IgM (Seth et al 1985).

Various workers also reported the reduced level of IgM in tuberculosis (Cherushenko et al 1982, Gupta et al 1983, Baganha et al 1983, Seth et al 1985 & 1987).

Seth et al in 1985 found decreased level of IgM in SMP and PPC group as compared to control. This reduction was significant in comarison to control.

Seth et al (1987) found that the level of IgM was consistently decreased in all the disease groups with highly significant reduction of TBM cases.

However, a number of workers have reported that the level of IgM was not markedly changed specially in pulmonary tuberculosis (Faulkner et al 1967, Jha et al 1974, Skvor et al 1979).

Singh et al (1984) reported higher level of IgM in pulmonary tuberculosis as compared to control. They opined that significant higher level of immunoglobulins (IgG, IgM and IgA) was a true reflection of humoral response in pulmonary tuberculosis. Okapai et al (1989) and Wong et al (1990) also reported raised IgM level in pulmonary tuberculosis. None of these workers studied IgM level in TBM cases.

Immunoglobulin G : (Table IX, X and XI)

In the present study, the mean level of IgG in PPC, PPD and TBM was 197.0 $\pm$ 65.22 IU, 189.05 $\pm$ 51.48 IU and 93.22 $\pm$ 20.02 IU respectively, while the IgG level in control group was 141.36 $\pm$ 27.66 IU.

It is evident that mean level of IgG was raised in PPC and PPD cases as compared to control though this difference was not significant statistically ($p > 0.05$). The mean level of IgG in TBM was decreased significantly in comparison to control cases ($p < 0.05$).

Humoral antibody production by B lymphocytes is substantially affected in tuberculosis. A gradient of the humoral antibody response was found in the present study with an increase of IgG concentration in all groups except TBM and decrease in IgM concentrations in all groups including TBM. The increased level of IgG in PPC and PPD cases demonstrated that there was adequate humoral immunity though nonspecific, while decreased IgG level in TBM demonstrated that there was lack of humoral response in TBM cases.

Significantly higher level of IgG in pulmonary tuberculosis has been reported by various workers (Faulkener et al 1967, Jha et al,1974, Skvor & Trinka 1979, Agnihotri et al 1978, Singh et al 1984, Seth et al 1985, Okapai et al 1989, Wong et al 1990, Kumar 1990).

Singh et al (1984) reported significant higher level of IgG in pulmonary tuberculosis as compared to control cases and opined that there was definite humoral

response in pulmonary tuberculosis. This indicates that raised antibody levels was reflection of antigenic load on the body presented by different antigenic components of mycobacterium.

Seth et al (1985) found raised IgG level in SMP and PPC cases as compared to control. They opined that raised IgG level was due to adequate humoral response in these two groups, resulting in milder form of disease.

Seth et al (1987) in another study found significantly raised IgG level in PPC and PPD groups while in TBM, IgG level was significantly reduced indicating severity of disease.

Kumar (1990) noted a rise in serum level of immunoglobulins in primary complex, however, observed that the rise in immunoglobulin level was statistically significant only in the vaccinated group. He also found decreased level of IgG in TBM and miliary tuberculosis, indicating severity of disease.

However, Murthy et al (1991) reported significantly raised serum and CSF, IgG, IgA and IgM levels in patient of TBM.

Correlation of serum Immunoglobulins in TBM and PPC and PPD groups : (Table XII & XIII)

In the present study, it was evident that all the immunoglobulins were lower in TBM cases when compared with the values of control. However, in the PPC and PPD cases IgG and IgA levels were raised in comparison to control.

Thus, reduced level of IgG and IgA in TBM was more significant when compared with PPC and PPD cases, because IgG and IgA levels were already raised in these groups.

Statistically, highly significant decreased levels of IgG and IgA was observed in TBM group, when compared with PPC and PPD respectively ($p < 0.05$).

Seth et al (1987) also reported that reduction in IgG level in TBM was even more significant when comparison was made with PPC and PPD cases.

Thus in nut-shell, the finding of present study reveal that no significant changes were observed in total proteins and fractions of various form of tuberculosis. All the three groups were statistically insignificant amongst themselves as well as to the values observed in control group of cases. The IgA level was increased in

PPC and PPD cases, however, it was reduced in TBM cases. The IgM level was decreased in all group of cases with significant reduction in TBM cases, indicating the severity of disease. IgG level was raised in PPC and PPD cases, however, IgG level was reduced significantly in comparison to control value. The reduction of IgG level in TBM was even more significant when comparison was made with PPC and PPD cases.

SUMMARY & CONCLUSION

SUMMARY AND CONCLUSION

The present study was conducted in Department of Paediatrics, M.L.B. Medical College, Hospital, Jhansi (U.P.) on 26 children of study group and 6 children of control group.

Children among study group were in the age group of 3-12 years. The maximum number of cases (46.15%) were seen in the age group 3-6 years followed by 30.76% in the age group of 6-9 years. The control group consisted of six healthy children in the age group of 3-12 years with almost similar pattern of age distribution. There was slight male prepondarence over female in study group.

Primary aim of this study was to findout humoral response (IgA, IgM and IgG) in various form of tuberculosis and its significance, if any, in serodiagnosis.

Three type of tuberculous cases were included in the study viz., primary pulmonary complex (PPC), progressive primary complex disease (PPD) and tubercular basal meningitis.

The selection of cases was done on clinical symptomatology viz., fever, cough, weight-loss, anorexia and failure to thrive. Mantoux test was performed in

each and every case. The induration of 710 mm was recorded as mantoux positive. The mantoux positivity was 100% and 98% in primary pulmonary complex (PPC) and progressive primary disease (PPD) respectively, while all the cases of TBM and control group were mantoux negative.

Complete haemogram and X-ray chest (PA view) were done in each and every case.

The maximum number of cases were seen in grade I and II malnutrition in the study and control group. There were only 3 cases of study group falling in grade III malnutrition, two had TBM and one case had PPC.

There was no control case having grade III malnutrition.

After taking a detailed history and doing clinical examination, total serum proteins, albumin, globulin and all the three major serum immunoglobulin levels were estimated by single radial immunodiffusion method of Mancini et al (1965). The observations thus collected were statistically analysed.

The haemoglobin level showed a decrease in patients with pulmonary form of tuberculosis and those with TBM. The lymphocytosis was more common in TBM as compared to control cases. ESR was raised significantly in all the clinical groups of patients. However, ESR was highly raised in TBM cases. Thus, estimation of ESR was found to be the indicator of the extent of disease, apart from radiological assessment.

Serum Proteins and fractions :

The present study revealed no significant changes in total proteins amongst the various group of tuberculosis viz., PPC, PPD and TBM and when compare to control group.

The serum albumin level revealed decreasing trend from mildest form to most severe form. However, no significant difference was observed amongst the various group of tuberculosis viz., PPC, PPD and TBM.

Thus serum albumin level was comparable to each others.

The serum globulin level was also comparable amongst various group of tuberculosis.

Serum Immunoglobulins :

The present study revealed that IgA level was increased in PPC and PPD cases while IgA level was reduced in TBM cases. However, no significant change in IgA level was observed in various group. IgA level was significantly reduced in TBM cases when comparison was made with PPC and PPD cases.

IgM level in present study showed decreased level in all forms of the disease viz., PPC, PPD and TBM. However, significant reduction was observed in TBM cases ($p < 0.05$). This significant decrease of IgM level in TBM, thus indicated the severity and chronicity of disease.

IgG level was higher in PPC and PPD cases. However, IgG level was significantly reduced in TBM cases when comparison was made with the control group ($p < 0.05$). IgG level in TBM cases showed a highly significant reduction when comparison was done with PPC, PPD cases, though the reduction was not as much significant ($P < 0.05$) when comparison was done with control cases. This was apparently due to rise in IgG level in PPC/PPD cases well above those seen in control, though not significant ($P > 0.05$).

The IgM level in the present study had consistently decreased in all form of tuberculosis with a significant reduction in TBM. The IgA level had increased in all groups of tuberculosis except TBM, where IgA level had decreased.

The increased level of IgG and IgA in tuberculosis and decreased level of IgM indicated a definite humoral response in tuberculosis. This increased level of immunoglobulins (IgG & IgA) also indicated adequate humoral immunity found in milder forms of disease.

Measuring specific antibody response in the assessment of severity of the disease and hence planning of drug therapy viz., two, three or four drugs regime.

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APPENDIX

APPENDIX

WORKING PROFORMA

STUDY OF SERUM PROTEINS AND IMMUNOGLOBULIN LEVEL IN CHILDHOOD TUBERCULOSIS

Case no.

MRD/OPD No. Dated

Patient's name : Age/Sex

Father's name :

Address :

Occupation :

Socio-economic status :

HISTORY :

I. Cough

a. Duration
b. With/withour expectoration
c. Amount of sputum
d. Mucoid/purulent/mucopurulent
e. Haemoptysis

II. Fever

a. Duration
b. Severity : Mild/moderate/High
c. Type : Continuous/intermittent/Remittent
d. Any associated factors

III. Appetite : Lost/Retained Duration

IV. Loss of weight : Duration

V. Failure to thrive : Yes/No Duration

VI. Headache/Vomiting : Yes/No

VII. Convulsions :

a. Duration
b. Type
c. Frequency
d. Associated factors

VIII. Consciousness : Impaired/Intact

IX. Breathlessness : Present/absent

a. Duration :
b. Onset :
c. Severity :

X. Chest pain : Present/absent

a. Duration : c. Onset :
b. Site : d. Relieving factor :

XI. Distension of Abdomen : Present/absent
Duration :

XII. Loose Motions : Duration :
Amount :
With/without mucous/blood/both

XIII. Bowel Movement : Constipation
Diarrhoea
Normal

XIV. Swelling over Neck : Yes/No

a. Duration: c. Discharge (if any) :
b. Pain : d. Association :

XV. Other complaints :

HISTORY OF PAST ILLNESS :

a. Measles/Pertusis/Exanthematous fever :
b. Malnutrition :
c. Others :

FAMILY HISTORY :

a. Suggestive of tuberculosis : Present/Absent
b. Others

DIETARY HISTORY :

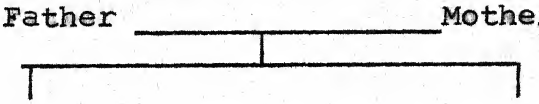
a. Calories : b. Protein :

IMMUNIZATION HISTORY : Immunized/Not immunized

a. DPT : b. BDG :
c. Polio: d. Measles :

GENEOLOGICAL HISTORY :

Father _____ Mother



GENERAL EXAMINATION :

G.C.	J.V.P.
Pulse rate	Others : Hairs
Heart rate	Fontanelle
Resp. rate	Frontal bossing
B.P.	Eyes :
Temperature	Oral cavity :
Pallor	Mucosal (Buccal)
Icterus	Teeth
Oedema (Pedal)	Lips
Clubbing	Skin :
Lymphadenopathy :	Subcutaneous tissue
-Cervical	Muscles :
-Inguinal	
-Axillary	

ANTHROPOMETRIC EXAMINATION

Height / Supine length	:	Weight :
Head circumference	:	MAC :
Chest circumference	:	

SYSTEMIC EXAMINATION

A. RESPIRATORY SYSTEM :

I. Inspection

Shape of chest :
Movement of chest :
Signs of respiratory distress :
Others :

II. II. Palpation :

Trachea : Central/Rt/Lt.
Movement of chest
Apex beat
Vocal Fremitus

III. Percussion : Impaired/Dull/Stonydull
Resonant/Hyper resonant

Area :

IV. Auscultation :
Type of breath sound : Bronchial/vesicular
Added sound :
Vocal Resonance :

B. CARDIOVASCULAR SYSTEM

I. Inspection

II. Palpation

III. Percussion

IV. Auscultation

C. ABDOMEN

I. Inspection :

II. Palpation :

III. Percussion :

IV. Auscultation:

D. CENTRAL NERVOUS SYSTEM

I. Higher Centres (Functions)

II. Cranial Nerves

III. Motor System

a. Bulk : b. Tone :
c. Power :

IV. Sensory system :

V. Reflexes :

	Right	Left
Superficial		
Abdomen		
Plantar		
Cremesteric		
Deep		
Biceps		
Triceps		
Supinator		
Knee jerk		
Ankle jerk		
Ankle clonus		

VI. Sign of meningeal irritation

VII. Gait

VIII. Cerebellar signs

E. MUSCULOSKELETAL SYSTEM

I. Spinal Deformity :
II. Others :

Provisional Diagnosis :

INVESTIGATIONS

A. Haemogram

1. Haemoglobin : Gm% 2. TLC : Cells/cmm.
3. DLC : P % L %, E %, M %
4. ESR : mm in first hour.

B. Mantoux Test

C. Chest skiagram (PA View)

D. C.S.F. Report

E. Serum Proteins

1. Total :
2. Serum Albumin :
3. Serum Globulin :

F. Immunoglobulins

IgG IU

IgM IU

IgA IU